Volume 5, issue 1, 2016 • e-ISSN: 1857-8187 • p-ISSN: 1857-8179

Review Article

Trichinelosis in Animals

Healthcare

Keywords: trichinosis, Trichinella larvae, tropism, trichinelloscopic examination, trypsine techniques, xenodiagnostic experiments, etc.

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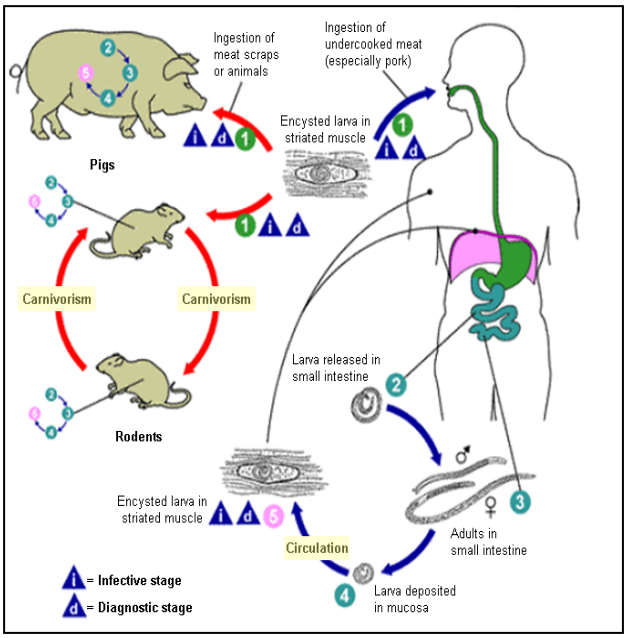
Abstract

Trichinellosis (also trichinosis) in animals is caused by nematodes (roundworms) of the family Trichinellidae Ward, 1970. Family characteristics. Parasites with small bud right body. They have not sexual pterygas. The anus is opened at the terminal part of body . The cloaks are opened at the ¼ frontal part of the body. The adult females are larva-productive. They are parasites of intestinal system. Trichinella spiralis (Trichina spiralis) Owen, 1833. Eight species of Trichinella are now recognize, based on host (Kapel, C M O 2000; Krivokapich, S J; Pozio E and D S Zarlenga, 2005; Pozio E et al, 1992), but the most important for animals domestic are: T. spiralis found as parasitic diseases on humans, pigs, rodents, and many carnivorous animals, of Europe, Asia, North America, with specific pathologies in pigs. T. nativae parasite of wild carnivorous of Euro – Asiatic areas norther of parallel 40°. It is specific diseases of carnivorous and omnivorous animals. T.nelsoni found as parasite of wilds carnivorous animals of Asiatic areas southern of parallel 40°. T. pseudospiralis is parasite of cats, rodents and pigs. It is recognized from other species because of the adult forms have smaller dimensions and forms non capsulated cists. T. spiralis is the cause of Trichinellosis, one of most important zoonosis allover the world. It is found worldwide in many carnivorous and omnivorous animals, insectivorous animals, rodents, wilds animals and humans (Pozio E and G Marucci 2003). It was found at 103 mammals. Occasionally may be found as parasite of horses. Developmental traits of T. spiralis is that infested hosts initially are final hosts because of they host adults forms at their intestine, but later on they are presented as intermediate host, because of they host larval forms at their muscles. Today's identification of samples to the species level and genotyping are based primarily upon molecular means (Pozio, E., and G. Marucci. 2003).

Morphology

Males are 1.5 mm long and with a diameter of 40µ. Females are 3 – 4 mm long and with a diameter of 60µ. Females have the genital pore near to the middle part of the body at the esophageal region. The body of Trichinellas is thin. They have specific trait, long esophagus covered by a layer of big cells, which have a glandular function. The larvae are formed after opening of eggs at the uterus and are with dimension 112µ. Meanwhile lavas muscles have the dimensions of 125-1000 µ, and the cists have the dimensions of 400–600–200–300 µ. The muscles cists can be seen with bare eyes. Inside of the cists, the larvae has the form of the letter U and S.At the same host are found also progressed larvae of the first stage. Biological cycle starts at the moment when these larvae together with the muscles which contain them are swallowed by one of the possible receptive hosts and spread at its intestine, some hours after they have reached there.



Biological cycle of T. spiralis.

Later on they have four moltings, and within a period of 2-3 days are changed at maturated males or females. The process of copulation happens at the intestine 40 hours after becoming infested, later on the males are dying, and females are introduced at the intestine mucosa, where are living there for a period of 4–16 weeks. During this period the females form the eggs, from which, inside the uterus appears larvae no longer than 0.1 mm, which move outside from the vulva. One female, while being alive can lay more than 1500 larvae.

Larvae entered at the lymphatic big vessels move towards the cava cranialis vein, and enter at the large blood circulation, which then spread at the entire body, but only the alive larva arrives to the striated muscles or stripped muscles. To the rest of the organs the larvae do not find normal good development conditions and so they are absorbed by the host's organism.

The muscles with highest larvae concentration are the diaphragm's pedicles, masticatory muscles, tongue muscles, laryngeal muscles, muscles of the eyes and intercostal muscles.

They penetrate into the interior muscle fibers, get a spiral form and are surrounded and encircled by a membrane which as a consequence of the reactivity of organs change into a cist with a form of a lemon and are found at the cross-transformed points from muscle fibers to the tendon fibers. They are completely developed from 6-8 weeks after infestation. Six months after cist appearance starts the calcification process. Larvae encapsulated in the interior of the cist may live nine months at the same host and even until 24 years.

The biological cycle starts again at the moment when the muscles are used as a food of carnivorous animals, omnivorous animals, insects, rodents, wild animals and humans. To humans, they are mostly widespread in the regions of North America and the Germany (probably from the unbaked well pig meat consummation) The reservoirs of the deceased are infested pigs and men. At the case that one carnivorous or omnivorous animal consumes meat with invasive larvae, they are fixed at the bunches of thin intestine for a period of 2-4 days till they reach adult developed stage. Thus, they traumatize intestine mucosa causing catarrhal inflammation and diarrhea. The toxins that originated from metabolic activity of larvae affect and influenc the thermoregulation center and consequently the body temperature increase. In the blood, there occur qualitative changes increasing the permeability of blood vase and pulmonary edema. From the larva localization in the muscle fibers, the fibers are damaged and so the myosites, muscular pain of rheumatoid arthritis and difficulty in swallowing appear.

Epidemiology

The *Trichinella* are present on all continents. Most of the species of *Trichinella* parasitizes predominantly the wild animals. A switch from wild animals to domestic animals can occur when there is an improper management in segregating husbandry and wildlife (Bruno Gottstein et al, 2009). The domestic or wild cycle can function independently or in cooperation with each other (Pozio, E and G Marucci 2003). The term "domestic cycle" refers to the transmission pattern where the focus is on a swine herd being fed, e.g., uncooked pork scraps, carrion, garbage, or the pigs can feed on carcasses that are not promptly removed from the farm; transmission can also become domestic via synanthropic animals living near the swine herd.

Regarding to the geographic distribution of the domestic cycle of *Trichinella*, since World War II, there have been no reports of infections on industrialized farms in Canada, the United States, and Western Europe. In several countries of Central-Eastern Europe, the transient breakdown of governmental veterinary services and state farms accompanied by economic problems and war have resulted in sharp increases in the incidence of *Trichinella* infection among domestic pig herds, with high prevalence in some villages in the 90s (Murrell K D and E. Pozio, 2000).

In the north America and most European Union countries, *Trichinella* infection in domestic animals has virtually disappeared, although sporadic foci do occur (Appleyard G D and A A. Gajadhar, 2000; Pozio E, P Cossu et al, 2008).

Trichinella infection is still endemic in Argentina, Chile, and Mexico in both humans and pigs (Ortega Pierres et al, 2000; Ribicich M, H R Gamble et al, 2005; Schenone, H., A. Olea, H. Schenone, M. C. Contreras, R. Mercado, L. Sandoval, and C. Pavletic. 2002). The domestic cycle of Trichinella occurs also in China (Wang Z Q, J Cui, and B. L. Xu. 2006). Foci of *Trichinella* infection of swine and humans also occur in Thailand, Indonesia, Laos, Malaysia and Myanmar (Pozio E, 2007). Natural *Trichinella* infections have been reported for more than 100 species of mammals, and in some species of birds and reptiles (Pozio E, 2005).

Despite the potential broad host spectrum for *Trichinella* spp., the predominant biotic potential concerns carnivores and porcine omnivores (Campbell W C, 1983). One of the most important biological factors promoting transmission is the physiological ability of musclestage larvae to survive in decaying carcasses/carrion. Encapsulated larvae of *T. spiralis* have been found to be infective for laboratory animals up to 4 months in extremely rotten meat (Madsen H. 1974.). The anaerobic metabolism favoring survival in putrefying flesh along with the ability of larvae of some species to survive freezing are two separate mechanisms that strongly increase the survival of the parasite in nature. It is important to stress that the survival of muscle larvae after freezing occurs mainly when these larvae parasitize striated muscles of carnivores, whereas the survival time after freezing is strongly reduced to a few days or weeks, when muscle larvae of the same strain parasitize other mammalian hosts such as swine and rodents.

Diagnosis

Direct Methods

Meat inspection for the detection of *Trichinella* larvae is designed to prevent clinical trichinellosis in humans but not to prevent infection. The identification of *Trichinella* larvae in muscle samples from pigs and other animal species intended for human consumption is limited to postmortem inspection of carcasses (Knapen K Noeckler, H Schenone and X. Zhu. 2000). Methods to detect *Trichinella* larvae in muscle samples need to be highly sensitive, and performance is greatly influenced by the sample size, the muscle type selected for sampling and the specific method used.

Clinic and Necropsies

It is difficult to be realized technically at the clinic practices. Clinically this can be realized only based at the clinic of hosted alive organisms. Are indentified and recorded during intestine phase also during muscular phase. The clinic suggests but do not confirm the diagnoses.

At the dead animals or butchered animals the diagnoses is determined by macroscopically examination of muscles, where it is observed tropism, by trichinelloscopic examination, by trypsine techniques or xenodiagnosis experiments.

Taking into consideration that the causal agent have the main natural host the pig and cause parasitic zoonosis, the diagnoses take a veterinary and human character. For veterinary physician take a big inters the control of pig meat control before consummation as food of humans.

The Artificial Digestion Method

One of diagnostic methods widely used for the detection of Trichinella larvae of is the artificial digestion method. It is using for a small number of samples, but it can be used also for more samples requests simultaneously.

The method of enrichment by artificial digestion for a restricted number of samples aims the enrichment of sample and is finalized by microscopically monitoring and trichinelloscopic examination.

The muscular samples are putted under artificial digestion at the funnel (Baermann's Apparatus), with pepsin 2-5 gr, pure HCl 10 cc and water 100cc. The Baermann's Apparatus is placed into at the temperature 37 – 40°C for 12 – 24 hours. Later on the muscular infiltrate is centrifuged. The liquid is removed and is examined the muscular precipitation. This method is more accurate then the method of trichinelloscopic examination, because of it reveals infestations at the situation the trichinelloscopic examination give negative results.

At same regions with endemic trichinellosis, have to be sterilized with frizzing or γ- ray of all pig meat carcasses.

The enrichment method through artificial digestion for a lot of requests samples is done simultaneously. First step is the digestion of meat at the acidified pepsin solution releases the alive larvae from muscular tissues. This general protocol can be used to discover infestation from trichinella at the meat.

Every method for detection of trichinella at the meat can be evaluated at the appropriate manner for its efficiency, in relation with known samples positive or negative, and later on can be monitored for its efficiency and effectiveness. The muscular samples have to be taken from places preferred from the larvae of trichinella species being tested. These regions are the diaphragm pedicles and the tongue for pigs, masseter muscles for horses. If the preferred regions of an animal species being tested are unknown at this situation is recommended to take samples from diaphragm and tongue.

The sample volume can be elected, in this manner, to reach the parameters of efficiency and sensitivity of the test: individual samples of 100 grams can be taken from an animal, or some samples can be taken from a group of animals, for creating a homogenous sample till 100 grams muscular tissues. The sensitivity of the test is reported to be in sample of 1 gram to distinguish the infestation of the level ∃3 larvae for one gram of tissues, and at the case of a sample of 3 grams to distinguish the infestation of the level ∃ 1.5 larvae for gram of tissues, and a samples of 5 grams to distinguish the infestation of the level ∃ 1 larvae for gram of tissues. Intended for public health, the testing of one gram muscular tissue of pig's meat (diaphragm and tongue), have been effective towards human trichinelloses reduction.

However, at the places to which the meat is supposed to be not fully cooked, or to be subjected to other after drying processing procedures, is recommended to be tested an appropriate number of samples to distinguish the level of infestation at least one larvae for a gram of tissues (for example a minimum sample of 5 grams).

The samples should be divested from adipose tissue and also from fasciates; because of this, tissues are undigested and do not have trichinella larvae. Later on the samples are mixed and softened to facilitate the digestion. The mixing is the method of selection. To prepare the samples through mixing, till 100 grams it is mixed with the same volume of acidified tap water (with 1% HCl) and is subject to shorter treatment (5–10 seconds) at a mixer of Warin type or Mulinet type.

A scarce mixing may result at the reduced digestion and a redundant mixing can result at the larvae destroying at the muscles' tissues. The mixing should continue until there are no visible meat parts. The meat preparation realized by meat gridding, till an acceptable method, taking into consideration that the size of holes must not exceed the diameter of 3 mm.

Artificial Digestion

Each 100 gram tissues must be digested at total volume of 1-3 liters acidified pepsin solution, using a estimated method. The ratio1:30 of meat for digestive solution (foe example 100 grams of meat have to be digested at a volume of 3 liters of digestive solution).

One must be careful at the moment of transferring of sample to its solution from mixer or grinder at the solution of volume 3-4 liters. Distilled pre-warmed water (45±2°C) acidified (0.5–1.0% HCl) witch have to be used for washing all parts including the blades of mixers or tiles of grinder later on pre-wormed acidified water have to be added till the appropriate volume (2-3 liters). Pepsin (1:10.000 strength of national standard form), have to be added to the mixture of acidified tap water till the ratio 0.5-1.0 weight/volume. At the case sample preparation with mixture of all the quantity of pepsin can be added to the initial mixture and be shaken to ensure the proper dispersion.

The mixture sample / pepsin has to be put at the container with 3–4 liters of water inside a vessel with volume 3-4 liters and be covered with alumina foils in order to prevent the clotting and it is steered with a steering magnetic plate (8–10 cm), or other alternative steering apparatus for a minimum time of 30 min (maybe a longer steering time will be needed to complete the digestion).

The temperature, during the digestion procedure has to be (45±2°C) and be under monitoring, using thermometer or other thermal registration apparatus. The temperature is monitored better in the case that we develop all the process in an incubator, a warming room, nevertheless a pre-wormed plate or a bain-marie are suitable substituents, if the temperature can be controlled within the prescribed limits. The digestion is considered to be completed if there do not exist parts of unsusceptible meat inside of digestive solution.

The Discovery of Larvae

At the end of digestive process, all the mixture is thrown and put at a baker through a metallic net (180 - 355 μm hole diameters) at a separating funnel of an appropriate size (2-4L), aided by a plastic funnel.

The baker and the metallic funnel have to be washed with an added volume (a minimum of 100 ml) tepid tap water. Not any undigested part of meat should not be seen in the net, If so, those parts have to be removed back to the digestive solution for more digestive procedures.

The mixture should be left at the separated funnel for a period of 30 minutes. A lot of options have to be taken into consideration for the clearness of samples. A volume of 40 ml liquid can be drained through the funnel, directly to the centrifugal tube of 50 ml. The content left for sedimentation for a period of 10 minutes, after all the content except 10 ml have to be sucked from the surface. In the case that residue10 ml are vague in a quantity of 30 ml warming tap water (37°C) have to be added to the sediment, and continue till the moment that sediment content is clearly visible. The last quantity of 10 ml will be taken for examination of *Trichinella's* larvae counting.

Trichinella spiralis

An alternative method for content clearing is to be used in another separating funnel of volume 125 ml liquid, and separating funnel into another funnel of volume 500 ml and the cont volume is adjusted for 500 ml with tap water at room temperature. This content is left for sedimentation for a period of 10 minutes. After sedimentation a sample of size 22 – 27 ml is taken for larvae counting. For both methods, it is important that the fluid needs to be removed from separating funnel with completed open valves. If the valves are partly open then this can result to the remaining of nematodes at the funnel.

Counting of Larvae

Aiming the counting of trichinella larvae, at the clear sediment, a drop is put at the Petri netted dish and examined for trichinellae larvae through a microscope with a magnification 15 – 40x. The liquid must be clear at a manner that does not have counting problems. In the case that the sediment liquid is not clear then it is necessary to be repeated the clearing procedure.

Molecular Techniques

For epidemiological studies and the improving of knowledge of the presence and the spread of *Trichinella spp* at the domesticated animals and wild animals, all the isolates have to be identified at the level of species and the genotype level. Since there are not morphological traits to identify these larvae, the molecular diagnoses are used to be selected diagnostic species or genotypes. With this scope is developed the multiple PCR technique, for simplifying and clearly differentiating species and genotypes of *Trichinella*. Data of partial sequences of DNA are generated at the internal transcript spacers for ITS1 and ITS2 regions of V segment of expansive repeated RNA from different species and genotypes of trichinella. This multiple PCR is a sensitive molecular method, it is a cheap and fast method with the capacity to identify without doubts and a sole larvae at the level of species and the genotypes.

Serologic Diagnoses

The animals can be tested for the presence of anti-trichinella anti-corps at the serum or the meat liquid, as during the live cycle (antemortum) also with postmortem examination. Nevertheless the serological method for trichinella is considered to be appropriate for the monitoring and epidemiological investigation research of domesticated animals and also wild animals. The time of seroconverting after primary infection from trichinella depending from the dose of infection and the muscular larvae loads is demonstrated from the complete comprehensive experimental researches with some species of trichinella and the different spectrum of host animals, such as: ping, horses, wild pigs and foxes. Longitudinal researches discovered that anti-corps and anti-trichinella can continue in pigs for a long for an unspecified time and it is supposed that at the moment of the pig slaughter it is not likely to have false or negative results, compared to another stage of infections because of hypothetic reduction of the level of anti-corps. Enzyme-linked immunosorbent assay (ELISA) is casually the most widely used method for discovering of trichinella infection, because of the sensitivity of this method, allowing the discovery and detection of one (1) larva for 100 grams muscular tissues (Office International des Epizooties, 2004). So it is performed a large series of experimental studies of this field using pig serum and samples of meat liquid. The specification capacity of ELISA is improved to a larger extent using metabolic antigens E/S which are released from trichinella larvae at the muscles also after in-vitro cultivation. A lot of experimental studies on the field ground are performed using pigs to evaluat ELISA sensitivity and efficiency using the E/S antigens. Multiple experimental studies are used on the field ground to evaluate the efficiency ELISA using E/S antigens. The status of infection to pigs is determined from digestion of samples of muscles and diaphragms. The sensitivity of ELISA ranges from 93.1 till 99.3%, while after examinations of serums of pigs the specificity ranged from 90.6 -99.4% and originated from free trichinella. In the studies for evaluation of

pigs using tyvelose antigens for ELISA, the sensitivity and specificity was respectively 94.3 and 96.7%, in comparison with antigen E/S ELISA, which had a sensitivity and specificity respectively 64.9% and 96.0%

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